Estimation of measurement uncertainty in food microbiology: *a normative approach*

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Bertrand LOMBARD

AFSSA-LERQAP, Maisons-Alfort, France

E-mail: b.lombard@afssa.fr

Outline

- The normative approach for food microbiology
- Measurement uncertainty in quantitative microbiology
- Measurement uncertainty in qualitative microbiology
- Interpretation against legal limits

Introduction

Why ?

- Accreditation of laboratories
  - Requirements of EN ISO 17025, § 5.4.6
    - To estimate the measurement uncertainty (MU) associated to the results produced by the laboratory
    - If rigourous/statistically valid calculation of MU not possible, to identify the MU components and to make a reasonnable estimation of them
  - Implementation in France (COFRAC) for food microbiology
    = delayed (see ISO works)

Why ? (foll.)

- Significance of microbiolgical analysis
  = direct hazard for the consumer health
- Quantitative methods in microbiology
  = highly variable
  → Need to quantify this variability
- How to express MU for qualitative determinations ?

The normative approach for food microbiology

The ISO structure

- ISO/TC 34/SC 9
  - TC 34 « Food products »
  - SC 9 « Microbiology »
- Microbiological analysis of foods & feeds
- « Horizontal » approach

Bangkok, December 2002

- MU in quantitative microbiology
  = Basic approach adopted
  → Technical Specification (ISO/TS)
    - Quicker publication
    - Users' review & 2-year revision
- MU for qualitative determinations
  = In a 2nd step

Measurement uncertainty in quantitative microbiology

The approach (1)

- Quantitative determinations
  - Colony-Count Techniques (+ Most Probable Number Techniques)
  - Alternatives methods (instrumental)
- Decision based on a 1st series of ISO trials (2002)
- Broad consensus at ISO meeting

The approach (2)

- GUM decomposition approach not selected
  - MU underestimation ?
  - Heavy in food microbiology

The approach (3)

- « Global » approach chosen
  - Reproducibility standard-deviation (s_R) on the final result of the entire measurement process
  - In agreement with
    - Codex Alimentarius (CCMAS)
    - ISO/TS 21 748 : 2004
      - « *Guide for use of the estimations of repeatability, reproducibility and trueness in the estimation of measurement uncertainty* »
      - Established by ISO/TC 69/SC 6
      - Bridge between GUM & ISO 5725
        ⇦ Global approach = special case of type A experimental estimation

The main steps

- 2003: 1st draft of ISO/TS 19036
  « *Microbiology of foods and animal feeding stuffs – Guide for the expression of measurement uncertainty of quantitative determinations* »
- 2003/2004: 2nd series of ISO trials
  - To quantify MU component linked to
    - Sampling of the test portion
    - Preparation of the initial suspension
  - 78 participants, from 10 countries
- 2004: Decisions for final draft of ISO/TS 19036
  - ISO Project Group
  - ISO/TC 34/SC 9

Guide ISO/TS 19036 - Presentation (1)

- Final Draft

  ↳ Under 3-month vote (→ 11 May) before publication

- Principles

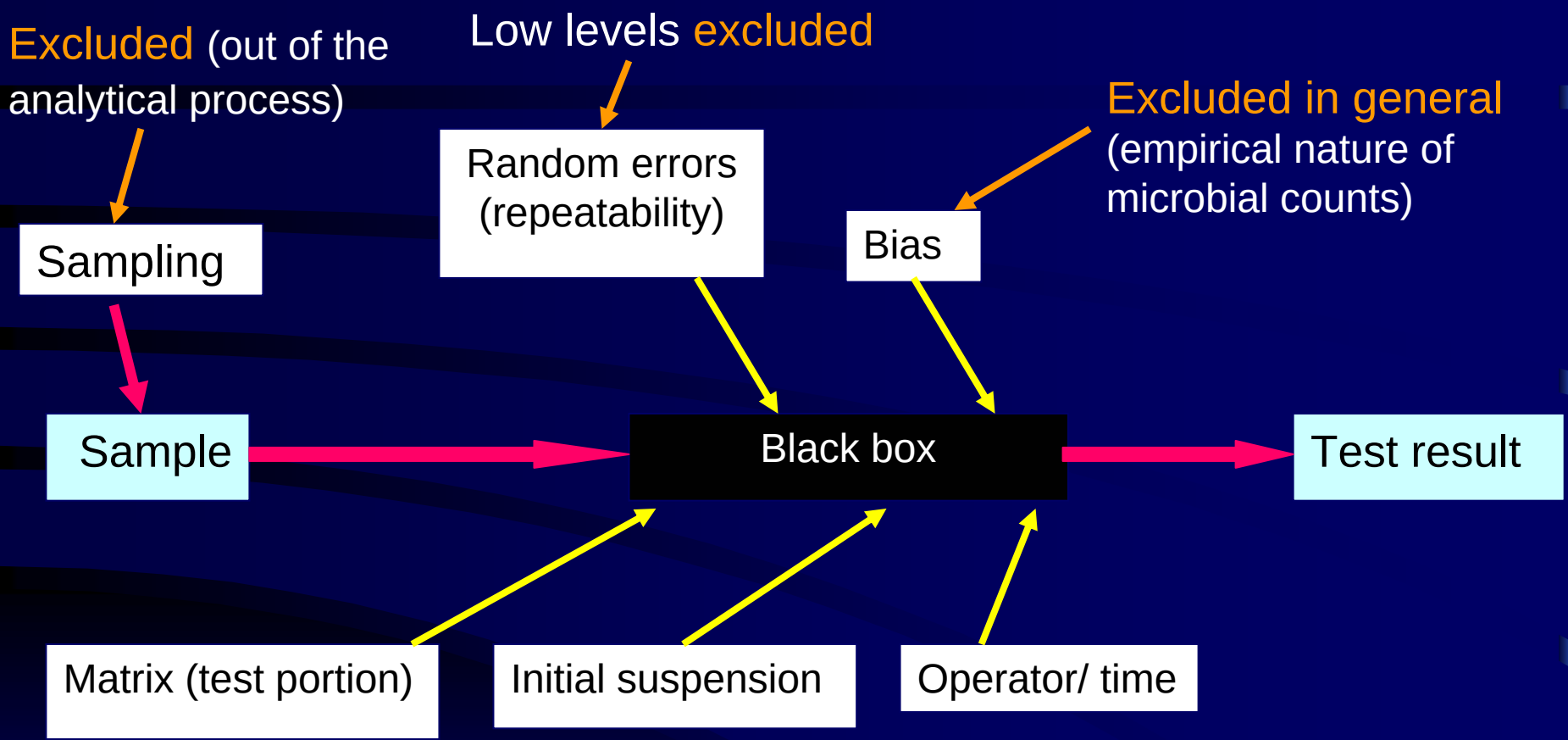
1. Global approach
2. Enlarged MU = 2 s_R

Guide ISO/TS 19036 - Presentation (2)

- Principles (foll.)

3. s_R estimated per
   - (consistent group of) microorganism(s)
   - (consistent group of) matrix(ces)
4. 3 options for s_R
   - Intra-laboratory s_R
   - Inter-laboratory s_R (method validation)
   - Inter-laboratory s_R (proficiency testing)

The « black-box » diagram

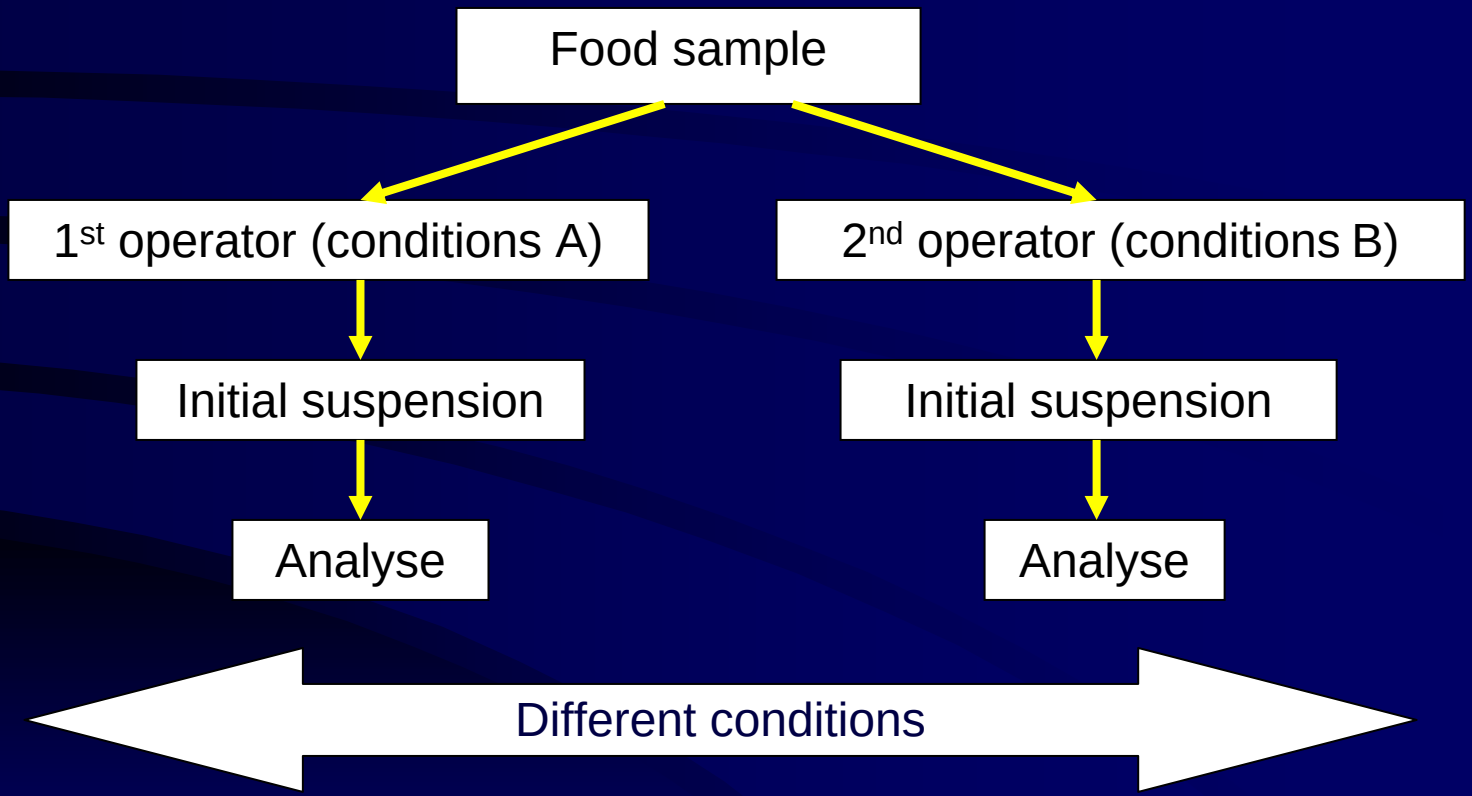


Intra-laboratory s_R (1)

- 1st (preferred) option
  - ⇘ linked to the laboratory *per se*

Intra-laboratory s_R (2)

Experimental protocol



Intra-laboratory s_R (3)

- The experimental protocol
  - 10 samples per matrix type
  - Advantage = MU « at large »
    - Heterogeneity of the sample contamination
    - Preparation of the initial suspension
  - Drawbacks
    - Need to repeat the protocol for each type of matrix analysed by the laboratory
    - Need to test naturally contamined samples

Inter-laboratory s_R (method validation) (1)

- Advantages = available values
- Drawbacks
  1. Conditions to meet (see ISO/TS 21 748)
     - Laboratory's bias & precision
       = compatible with the method's ones
     - All uncertainty sources (incl. test portion, sample preparation)
       = taken into account in the inter-laboratory trial

Inter-laboratory s_R (method validation) (2)

- Drawbacks (foll.)
  2. Inter-laboratory s_R available for a limited number of methods in food microbiology
  3. Difficulty
     - ➢ to apply to routine analyses
     - ➢ precision data obtained in limited and artificial conditions
       - – Combinaisons (matrix, strain)
       - – Annex flora (if any)
  4. Risk to under-estimate MU

     (samples homogenized and stabilized)

Inter-laboratory s_R (PT)

- Advantages
  - Available values
  - Large number of PT schemes in food microbiology
- Drawbacks
  1. Conditions to meet
     - Method used by the laboratory in PT = the same than in routine
     - Method used by a sufficient number of participants
     - PT samples ≈ routine samples
  2. Samples

Measurement uncertainty in qualitative microbiology

- Preliminary works at ISO
- 2 possibilities foreseen
  - CI around the Limit of Detection (LoD_{50})
  - From the equivalent of reproducibility for qualitative determinations

Interpretation against legal limits

Legislative frame

- Food hygiene « package »
  ↳ Regulation 852/2004 (« H I »)
- Draft Regulation on microbiological criteria (SANCO/4198 rev. 14, Dec. 2004)
- A microbiological criterion
  = a qualitative/quantitative limit +…

Food hygiene controls

- For operators' own checks
  = MU not taken into account
- For official food controls
  = ?
  - ➢ the same rule
  - ➢ or (Figure 2)
    - o case (ii) for indicators
    - o case (iv) for pathogens
  - → To be precised into EC Guidelines, to come

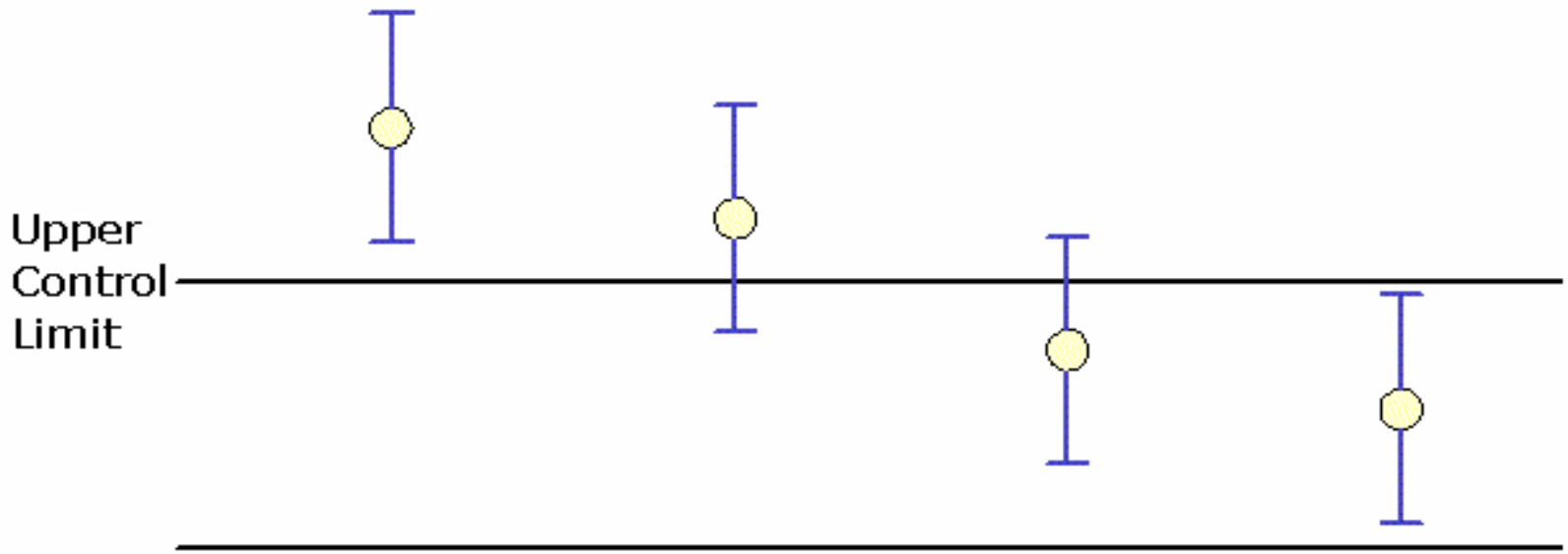


Figure 2: Uncertainty and compliance limits

Conclusion

- Global approach
  - Pragmatic, adapted to the complexity of
    - Food analysis
    - Microbiological analysis
  - In agreement with
    - GUM principles
    - International references and rules
- MU estimation becoming widespread in food microbiology
  = Towards a more « scientific » analysis ?